# BSC 4934: Q'BIC Capstone Workshop


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http://www.cs.fiu.edu/~giri/teach/BSC4934_Su10.html

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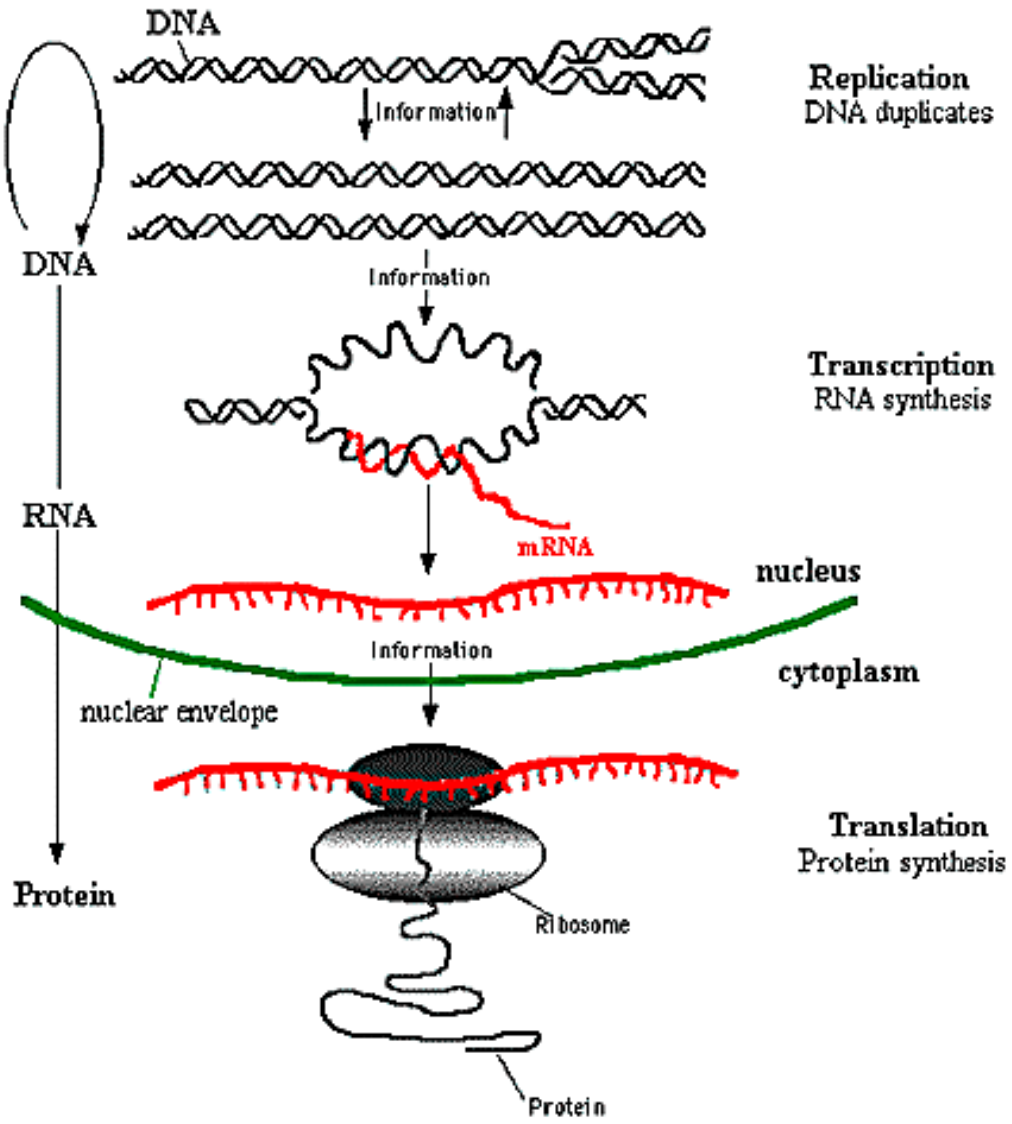
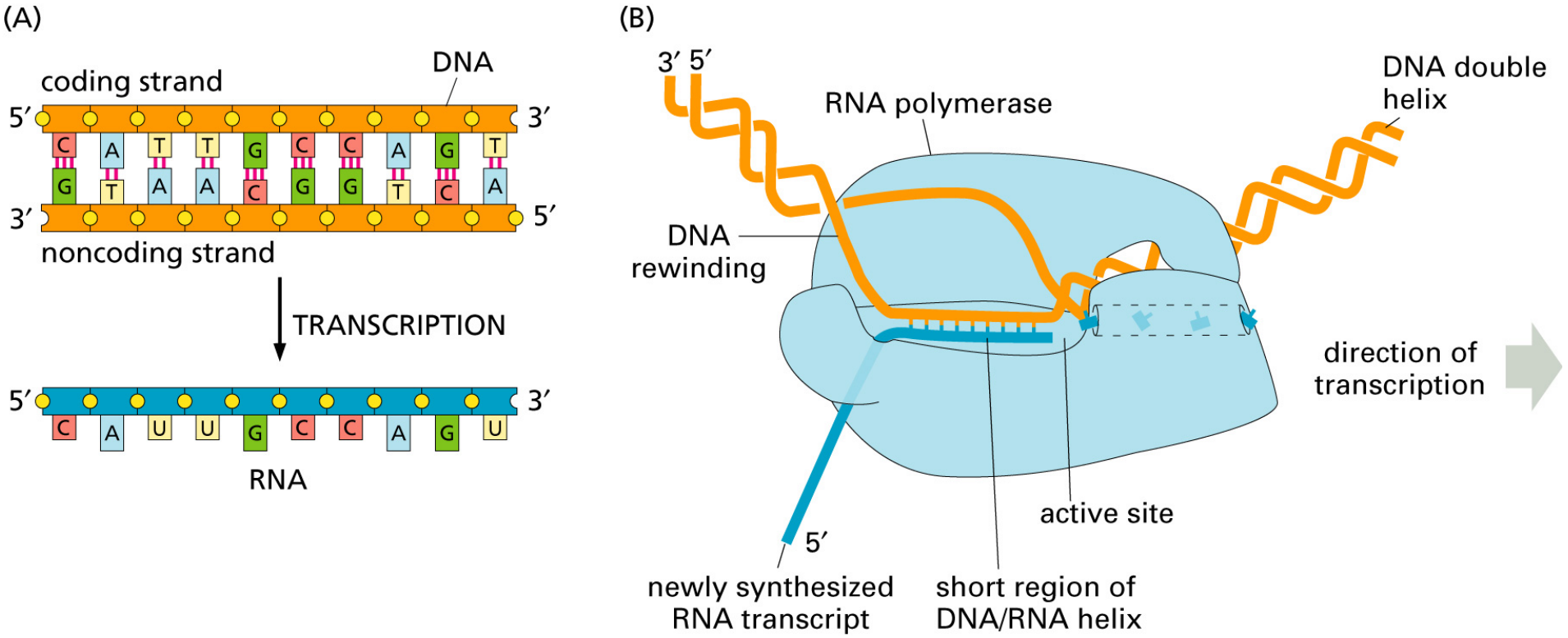
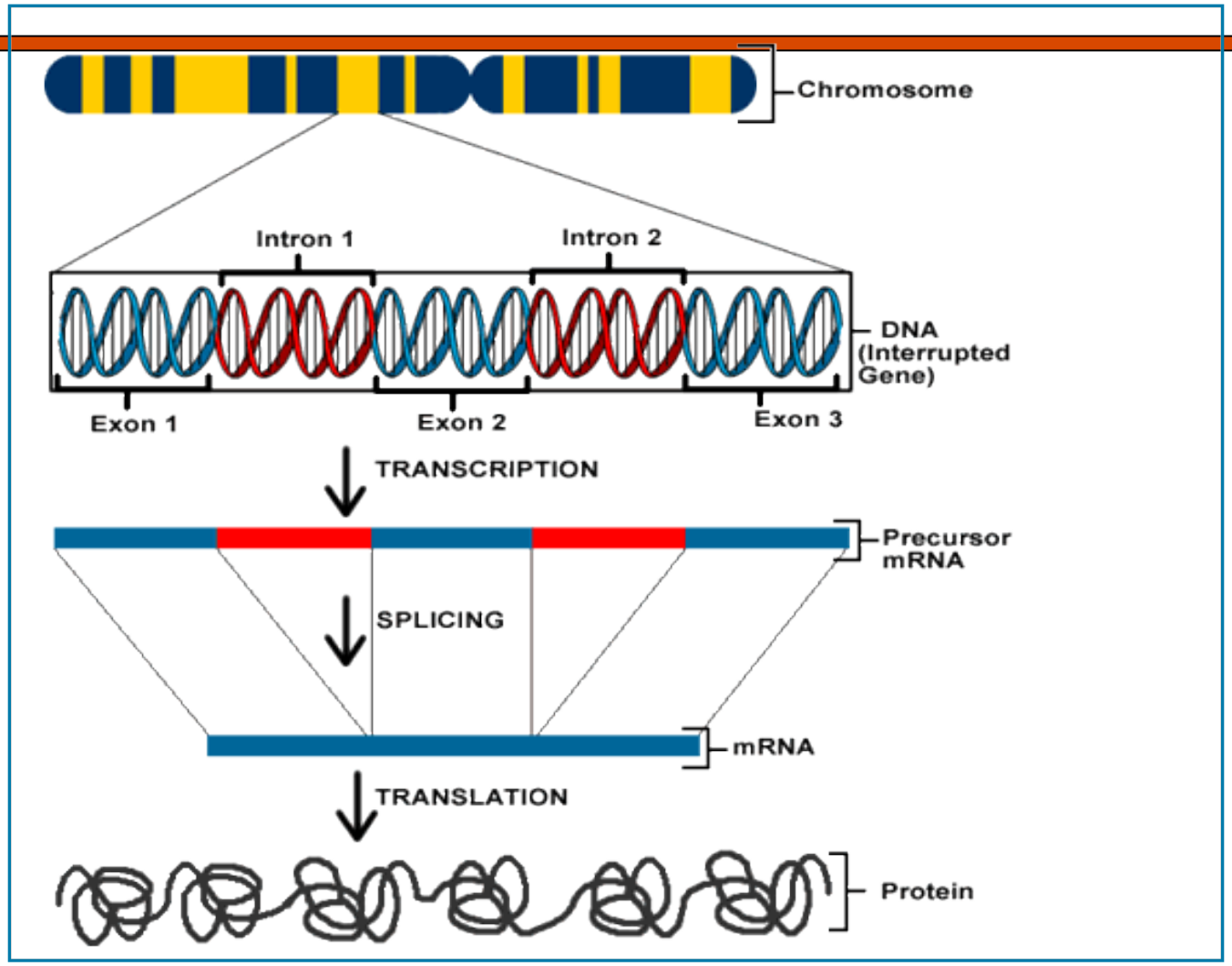



**The Central Dogma of Molecular Biology**

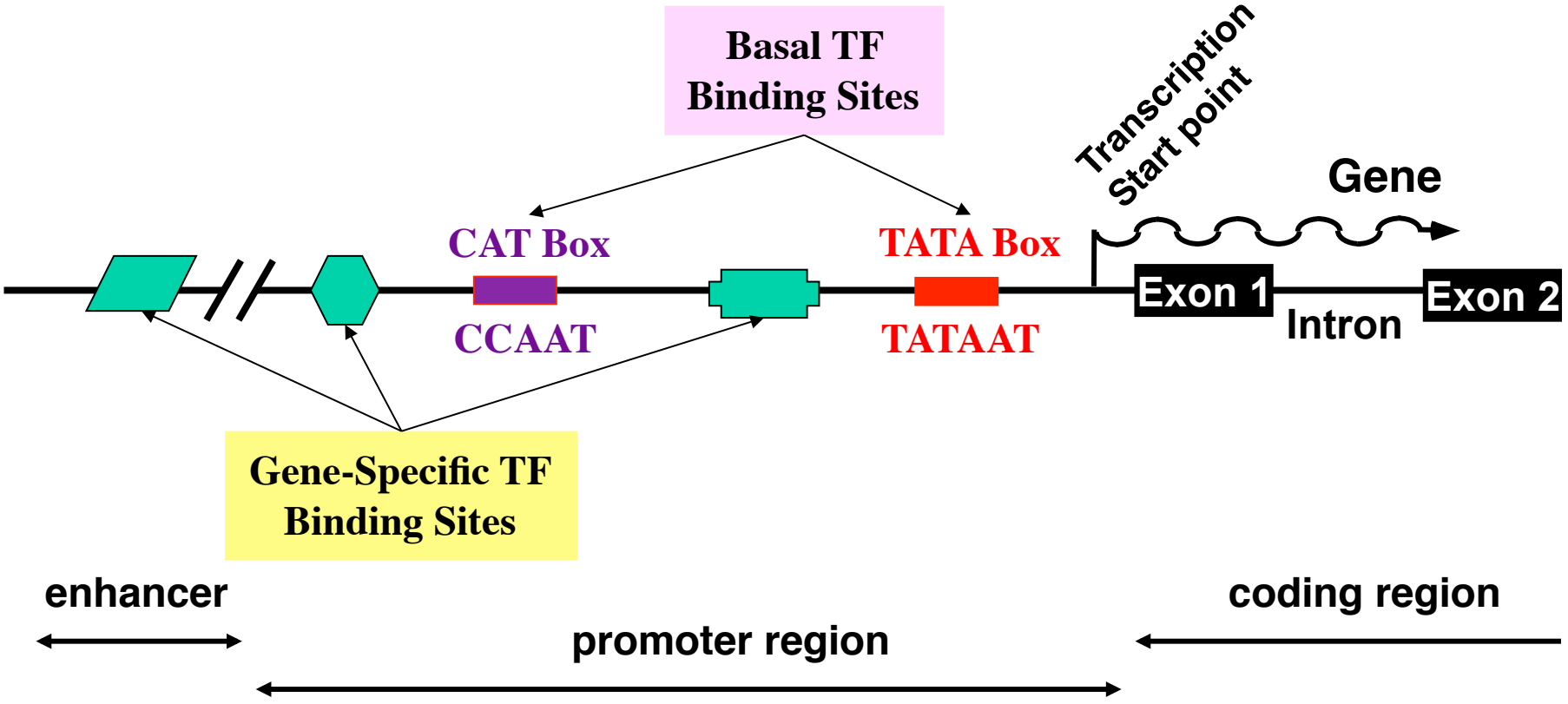
# Transcription

Fig 1.7, Zvelebil/Baum




Chromosome
Intron 1
Intron 2
DNA
(Interrupted
Gene)
Exon 1
Exon 2
Exon 3
TRANSCRIPTION
Precursor
mRNA
SPLICING
mRNA
TRANSLATION
Protein

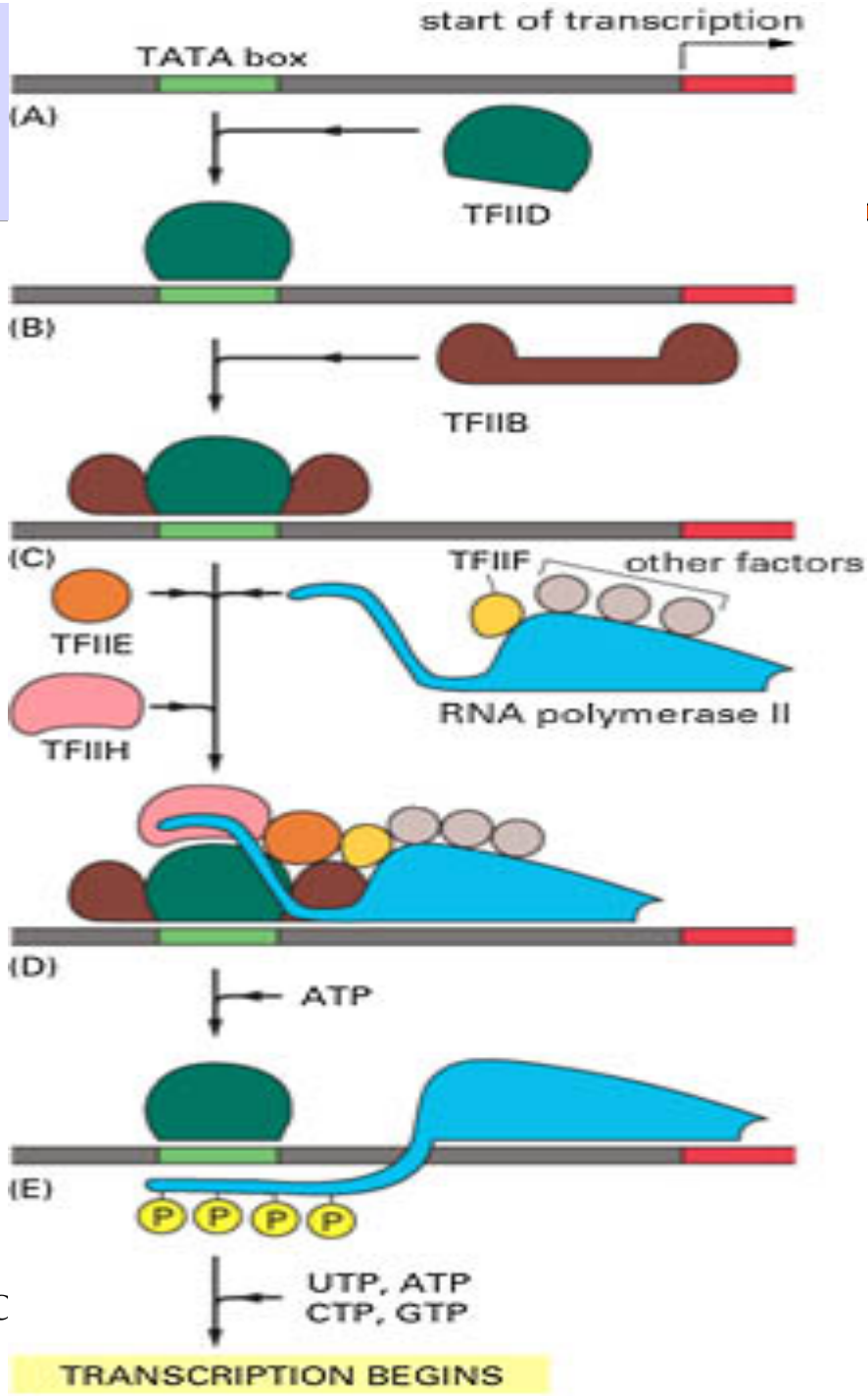
# Transcription Regulation






Courtesy: Kalai Mathee

# Transcription Initiation



# Transcription

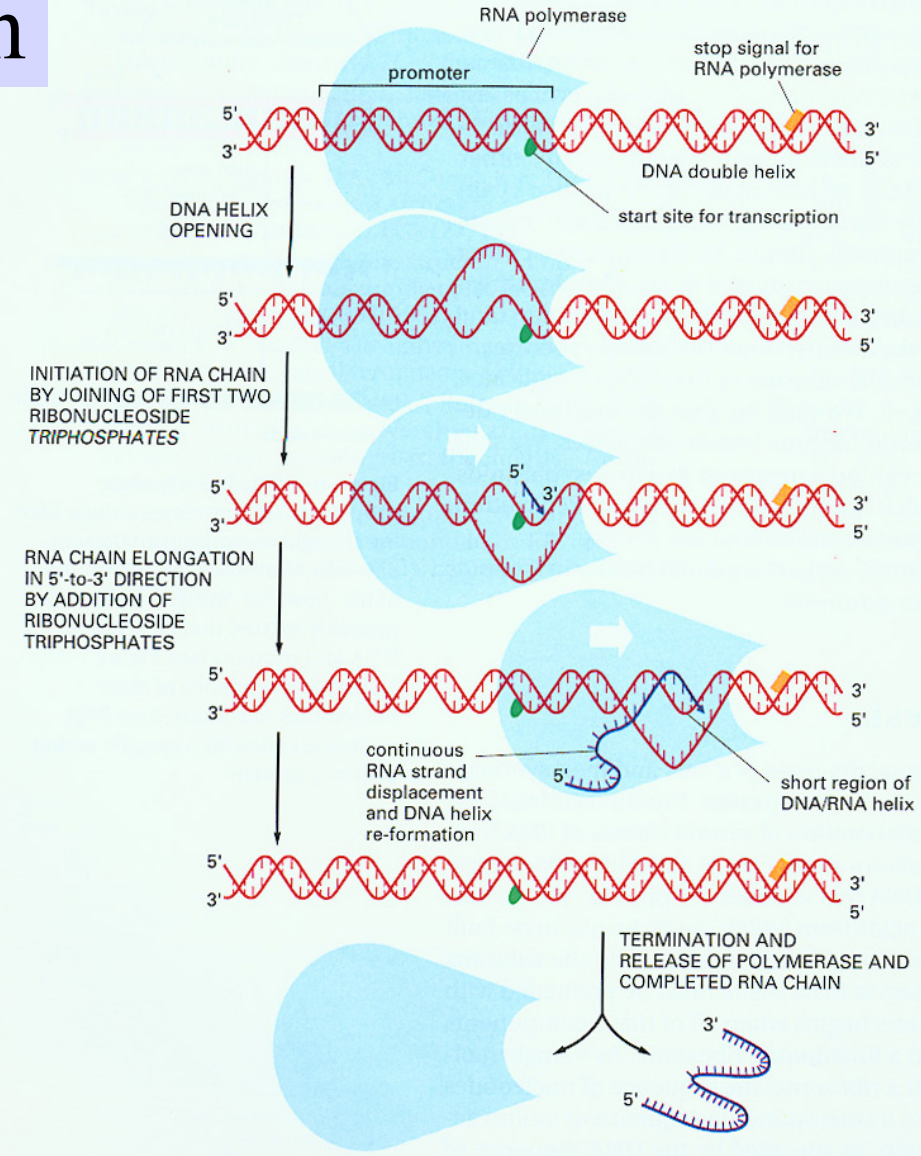


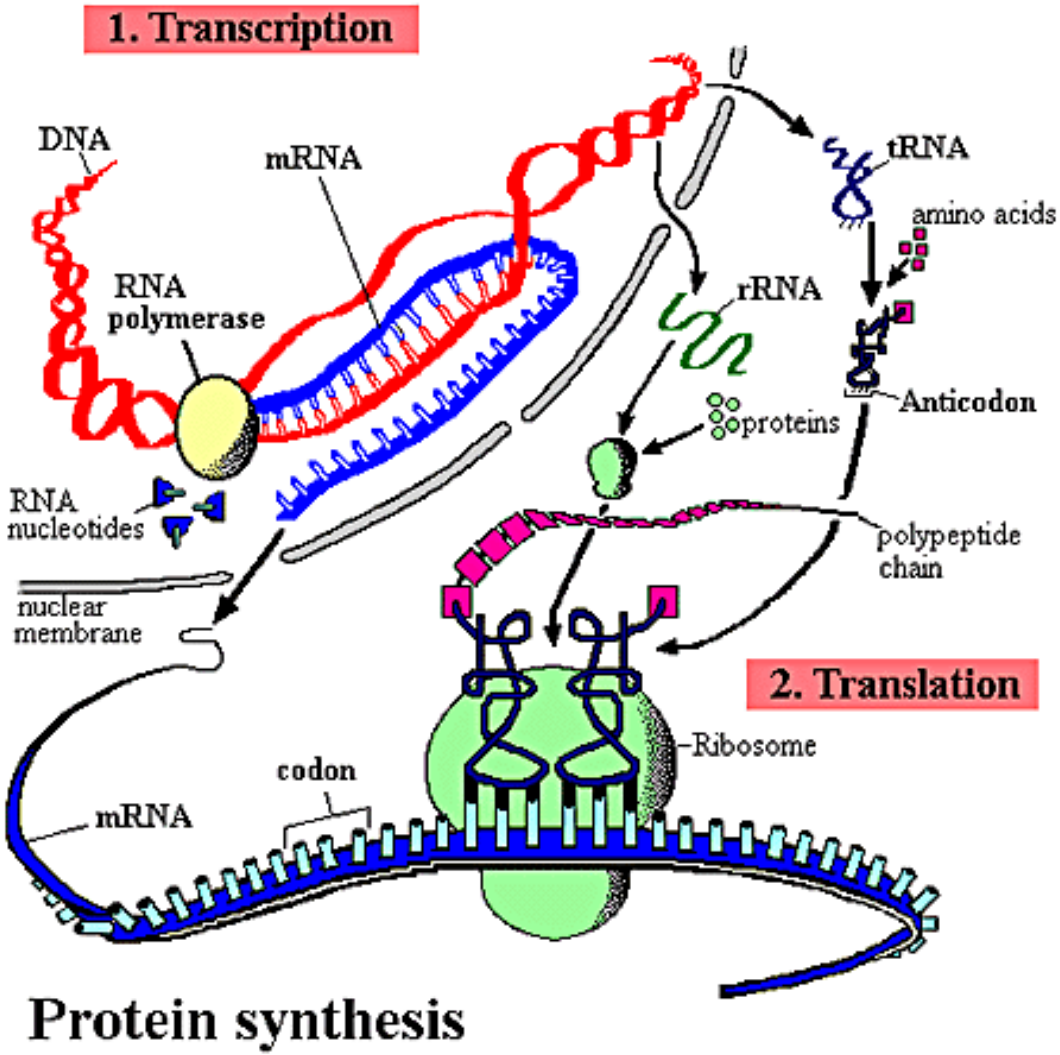

**Figure 6–2 The synthesis of an RNA molecule by RNA polymerase.** The enzyme binds to the promoter sequence on the DNA and begins its synthesis at a start site within the promoter. It completes its synthesis at a stop (termination) signal, whereupon both the polymerase and its completed RNA chain are released. During RNA chain elongation, polymerization rates average about 30 nucleotides per second at 37°C. Therefore, an RNA chain of 5000 nucleotides takes about 3 minutes to complete.

224 Chapter 6 : Basic Genetic Mechanisms

# Transcription Factors

- The general transcription factors have been highly conserved in evolution; some of those from human cells can be replaced in biochemical experiments by the corresponding factors from simple yeasts.

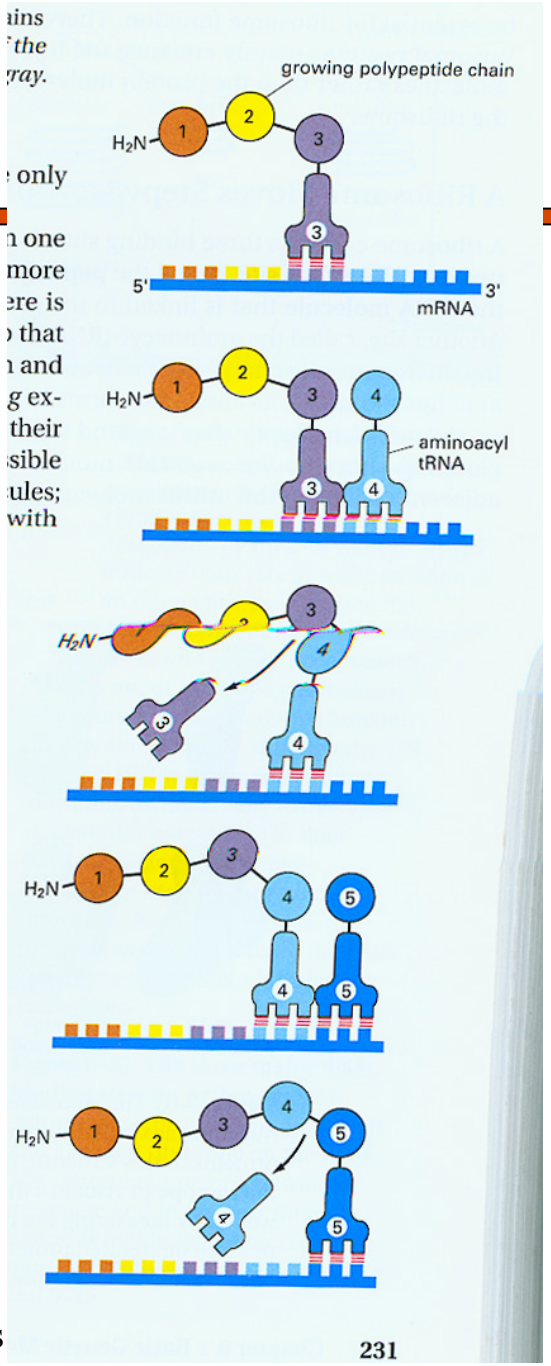
# Protein Synthesis

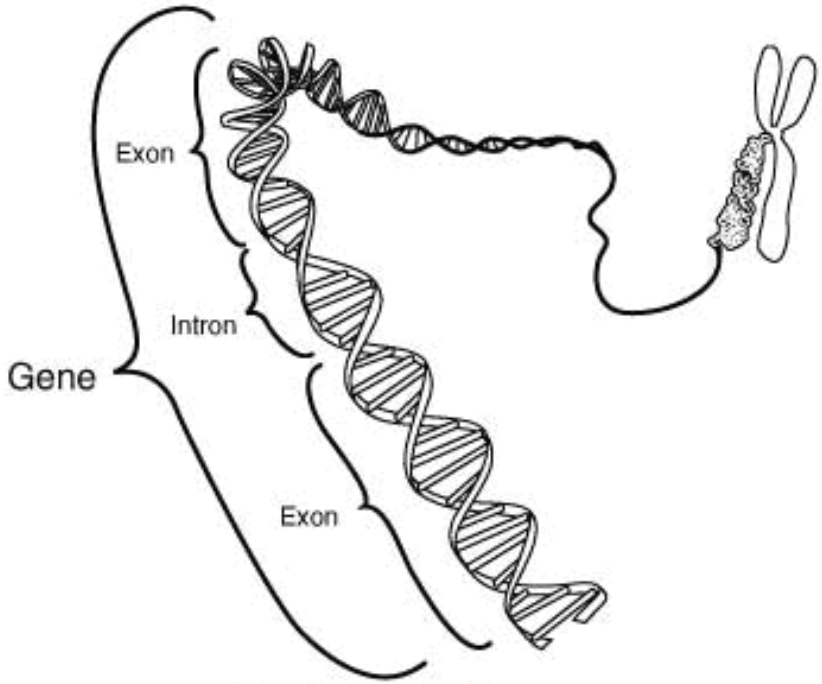


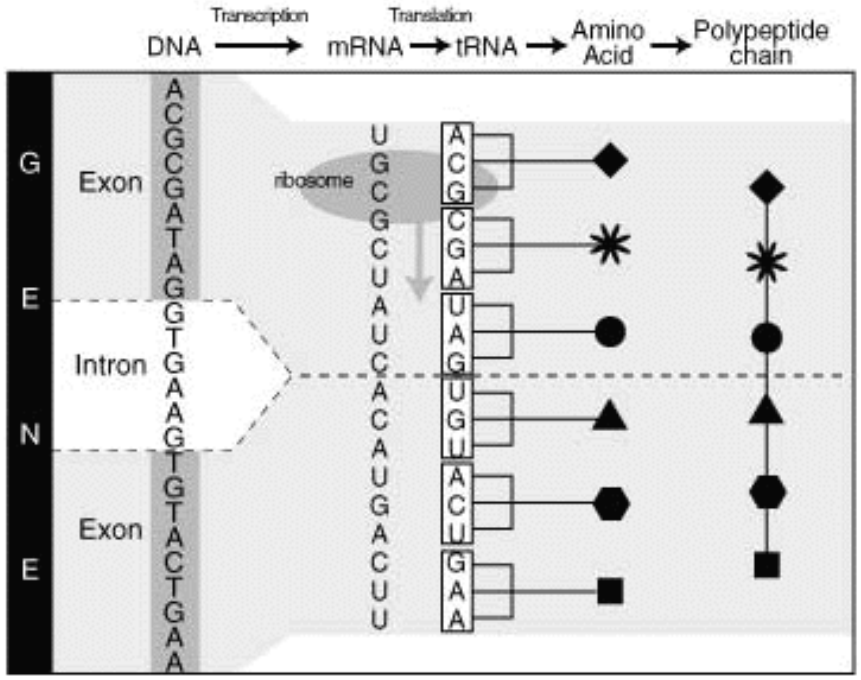

**Protein synthesis**

# Protein Synthesis:

## Incorporation of amino acid into protein




Exon
Intron
Gene
Exon


Transcription
Translation
DNA
mRNA
tRNA
Amino Acid
Polypeptide chain
G
E
N
E
Exon
Intron
Exon
ACGCGATAGGTGAAGTGTACTGAA
ribosome
UGCGCUAUCCACAUGACUU
ACG
CGA
UAG
UGU
ACU
GAA

# Drosophila Eyeless vs. Human Aniridia

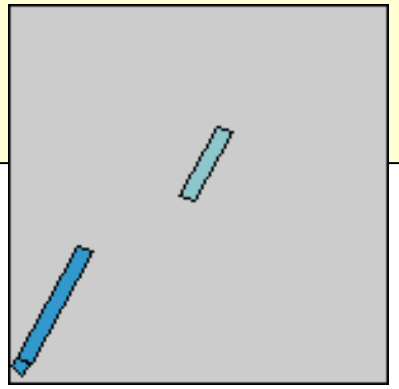
```
Query: 57   HSGVNQLGGVFVGGRPLPDSTRQKIVELAHSGARPCDISRILQVSNGCVSKILGRYYETG 116
            HSGVNQLGGVFV GRPLPDSTRQKIVELAHSGARPCDISRILQVSNGCVSKILGRYYETG
Sbjct: 5    HSGVNQLGGVFVNGRPLPDSTRQKIVELAHSGARPCDISRILQVSNGCVSKILGRYYETG 64

Query: 117 SIRPRAIGGSKPRVATAEVVSKISQYKRECPSIFAWEIRDRLLQENVCTNDNIPSVSSIN 176
            SIRPRAIGGSKPRVAT EVVSKI+QYKRECPSIFAWEIRDRLL E VCTNDNIPSVSSIN
Sbjct: 65   SIRPRAIGGSKPRVATPEVVSKIAQYKRECPSIFAWEIRDRLLSEGVCTNDNIPSVSSIN 124

Query: 177 RVLRNLAAQKEQ 188
            RVLRNLA++K+Q
Sbjct: 125 RVLRNLASEKQQ 136
```

```
Query: 417 TEDDQARLILKRKLQRNRTSFTNDQIDSLEKEFERTHYPDVFARERLAGKIGLPEARIQV 476
           +++ Q RL LKRKLQRNRTSFT +QI++LEKEFERTHYPDVFARERLA KI LPEARIQV
Sbjct: 197 SDEAQMRLQLKRKLQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQV 256

Query: 477 WFSNRRAKWRREEKLRNQRR 496
           WFSNRRAKWRREEKLRNQRR
Sbjct: 257 WFSNRRAKWRREEKLRNQRR 276
```



E-Value = 2e-31

# Implications of Sequence Alignment

- Mutation in DNA is a natural evolutionary process. Thus sequence similarity may indicate common ancestry.
- In biomolecular sequences (DNA, RNA, protein), high sequence similarity implies significant structural and/or functional similarity.

# Discovery based on alignments

- Early 1970s: Simian sarcoma virus causes cancer in some species of monkeys.
- 1970s: infection by certain viruses cause some cells in culture (in vitro) to grow without bounds.
  - Hypothesis: Certain genes (oncogenes) in viruses encode cellular growth factors, which are proteins needed to stimulate growth of a cell colony. Thus uncontrolled quantities of growth factors produced by the infected cells cause cancer-like behavior.
- 1983:
  - The oncogene from SSV called v-sis was isolated and sequenced.
  - The partial amino-acid sequence for platelet-derived growth factor (PDGF) was sequenced and published. It stimulates the proliferation of normal cells.
  - R.F. Doolittle was maintaining one of the earliest home-grown databases of published amino-acid sequences.
  - Sequence Alignment of v-sis and PDGF showed something surprising.

# PDGF and v-sis

- One region of 31 amino acids had 26 exact matches
- Another region of 39 residues had 35 exact matches.
- Conclusion:
  - The previously harmless virus incorporates the normal growth-related gene (proto-oncogene) of its host into its genome.
  - The gene gets mutated in the virus, or moves closer to a strong enhancer, or moves away from a repressor.
  - This causes an uncontrolled amount of the product (the growth factor, for example) when the virus infects a cell.
- Several other oncogenes known to be similar to growth-regulating proteins in normal cells.

# V-sis Oncogene - Homologies

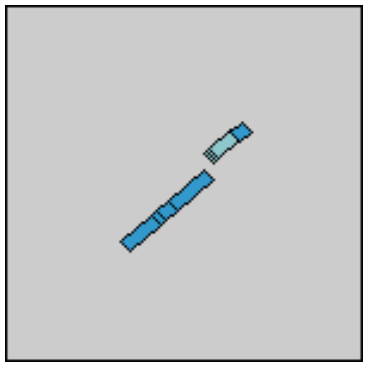
| Sequences producing significant alignments: | Score (bits) | E Value |
|---|---|---|
| gi\|332623\|gb\|J02396.1\|SEG_SSVPCS2 Simian sarcoma virus v-si... | 4591 | 0.0 |
| gi\|61774\|emb\|V01201.1\|RESSV1 Simian sarcoma virus proviral ... | 4504 | 0.0 |
| gi\|332622\|gb\|J02395.1\|SEG_SSVPCS1 Simian sarcoma virus LTR ... | 1283 | 0.0 |
| gi\|885929\|gb\|U20589.1\|GLU20589 Gibbon leukemia virus envelo... | 1140 | 0.0 |
| gi\|4505680\|ref\|NM_002608.1\| Homo sapiens platelet-derived g... | 954 | 0.0 |
| gi\|20987438\|gb\|BC029822.1\| Homo sapiens, platelet-derived g... | 954 | 0.0 |
| gi\|338210\|gb\|M12783.1\|HUMSISPDG Human c-sis/platelet-derive... | 954 | 0.0 |

# Sequence Alignment

```
>gi|4505680|ref|NM_002608.1|    Homo sapiens platelet-derived growth
   factor beta polypeptide (simian sarcoma viral (v-sis) oncogene
   homolog) (PDGFB), transcript variant 1, mRNA Length = 3373 Score = 954
   bits (481), Expect = 0.0 Identities = 634/681 (93%), Gaps = 3/681 (0%)
   Strand = Plus / Plus
Query: 1015 aggggaccccattcctgaggagctctataagatgctgagtggccactcgattcgctcct 1074
           |||||||||||||||| |||||||| ||| |||||||||||| ||||||||| |||||||
Sbjct: 1084 aggggaccccattcccgaggagctttatgagatgctgagtgaccactcgatccgctcct 1143
      > 21 E  G  D  P  I  P  E  E  L  Y  E  M  L  S  D  H  S  I  R  S
Query: 1075 tcgatgacctccagcgcctgctgcagggagactccggaaaagaagatggggctgagctgg 1134
           | ||||| ||||| |||||||||| |||||| ||||| | |||||||||| ||| |||
Sbjct: 1144 ttgatgatctccaacgcctgctgcacggagaccccggagaggaagatggggccgagttgg 1203
     > 61 D  L  N  M  T  R  S  H  S  G  G  E  L  E  S  L  A  R  G  R
```

# Sequence Alignment

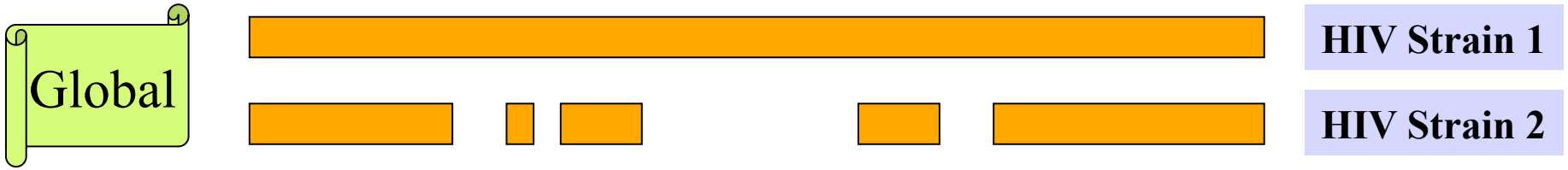
**Sequence 1 gi 332624 Simian sarcoma virus v-sis transforming protein p28 gene, complete cds; and 3' LTR long terminal repeat, complete sequence. Length 2984 (1 .. 2984)**
**Sequence 2 gi 4505680 Homo sapiens platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog) (PDGFB), transcript variant 1, mRNA Length 3373 (1 .. 3373)**



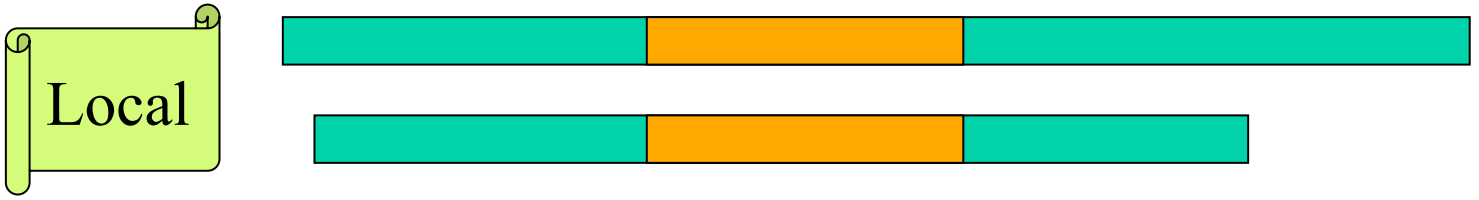
# Similarity vs. Homology

- Homologous sequences share common ancestry.
- Similar sequences are "near" to each other by some appropriately defined measurable criteria.

# Types of Sequence Alignments - 1

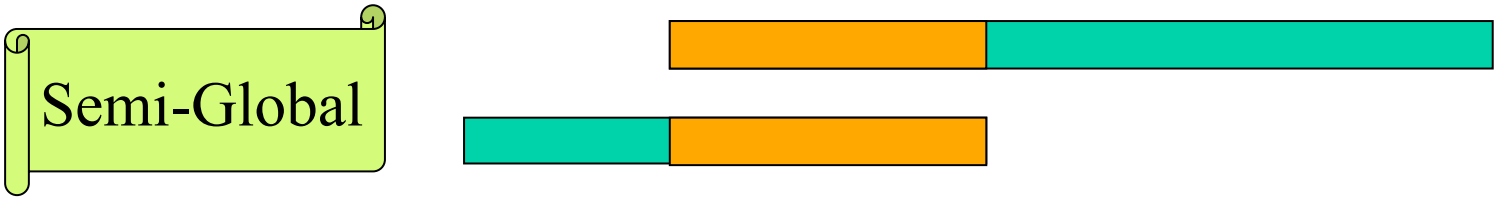



- Global Alignment: similarity over entire length

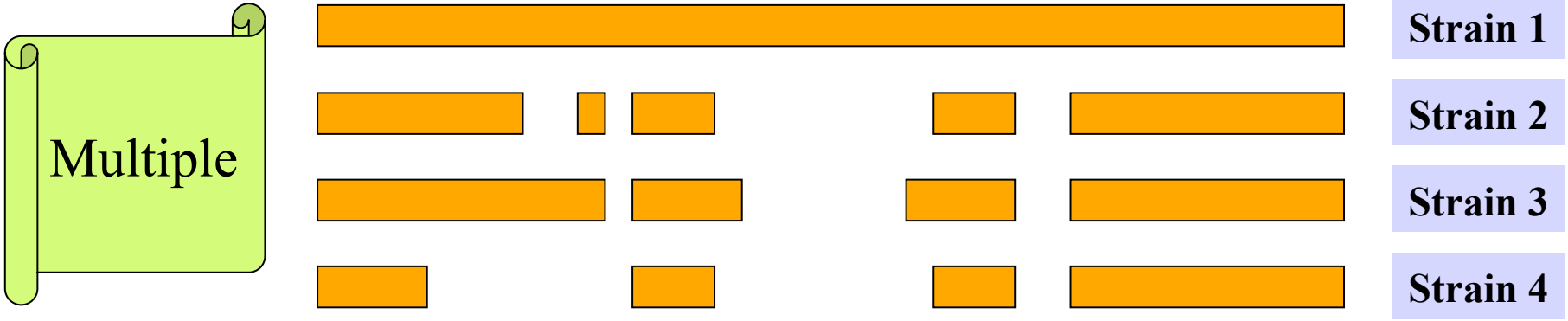



- Local Alignment: no overall similarity, but some segment(s) is/are similar

# Types of Sequence Alignments - 2




- Semi-global Alignment: end segments may not be similar



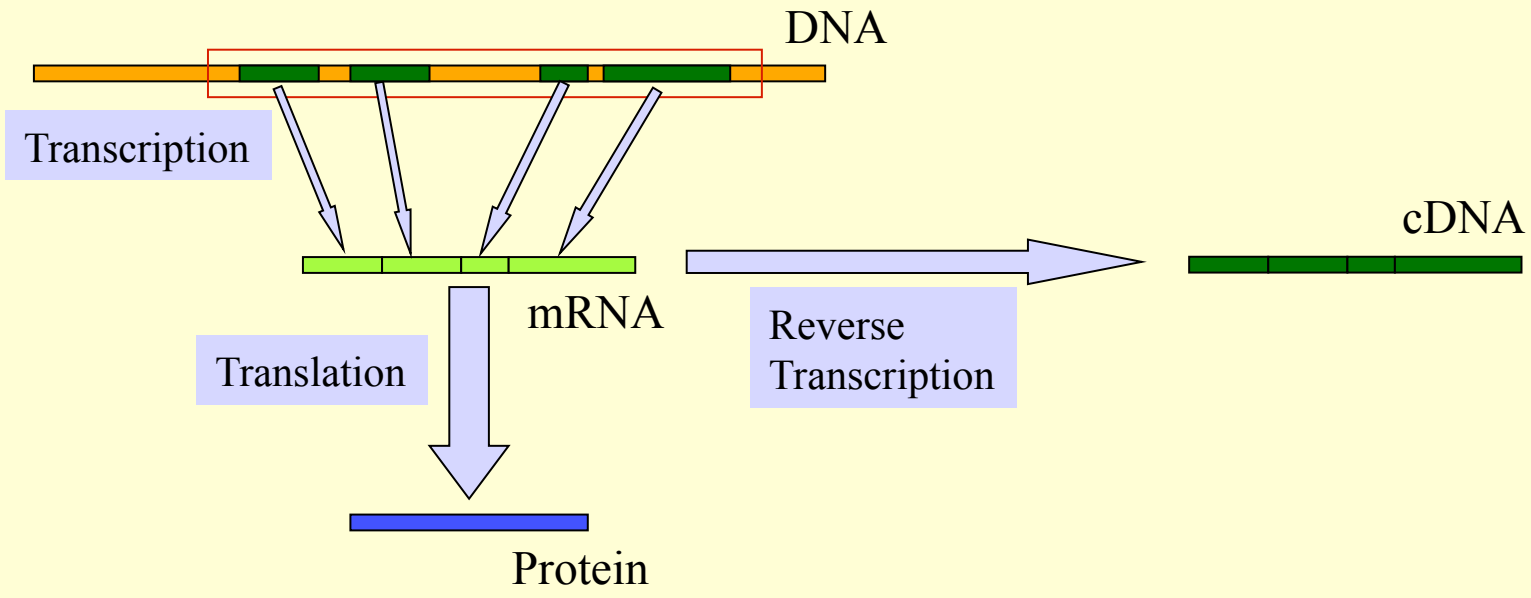

- Multiple Alignment: similarity between sets of sequences

# Sequence Alignment

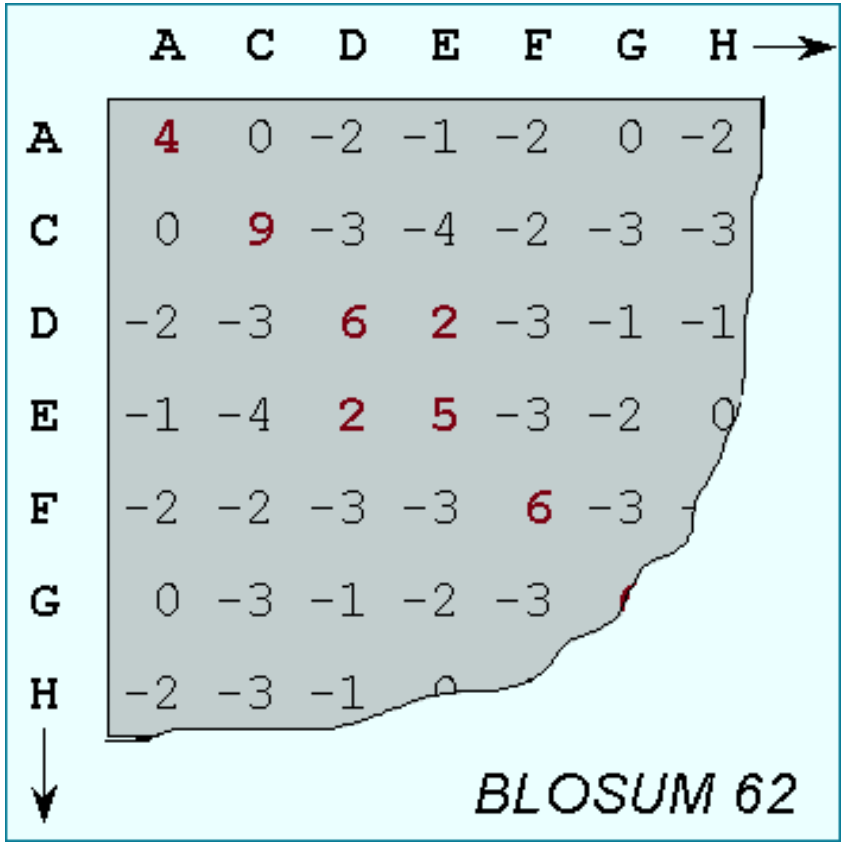
- Global:
  - Needleman-Wunsch-Sellers (1970).
- Local:
  - Smith-Waterman (1981)
  - Useful when commonality is small and global alignment is meaningless. Often unaligned portions "mask" short stretches of aligned portions. Example: comparing long stretches of anonymous DNA; aligning proteins that share only some motifs or domains.
- Dynamic Programming (DP) based.

# Why gaps?

- Example: Finding the gene site for a given (eukaryotic) cDNA requires "gaps".
- What is cDNA? cDNA = Copy DNA



# How to score mismatches?



# BLAST & FASTA

- FASTA

  [Lipman, Pearson '85, '88]

- Basic Local Alignment Search Tool

  [Altschul, Gish, Miller, Myers, Lipman '90]

# BLAST Overview

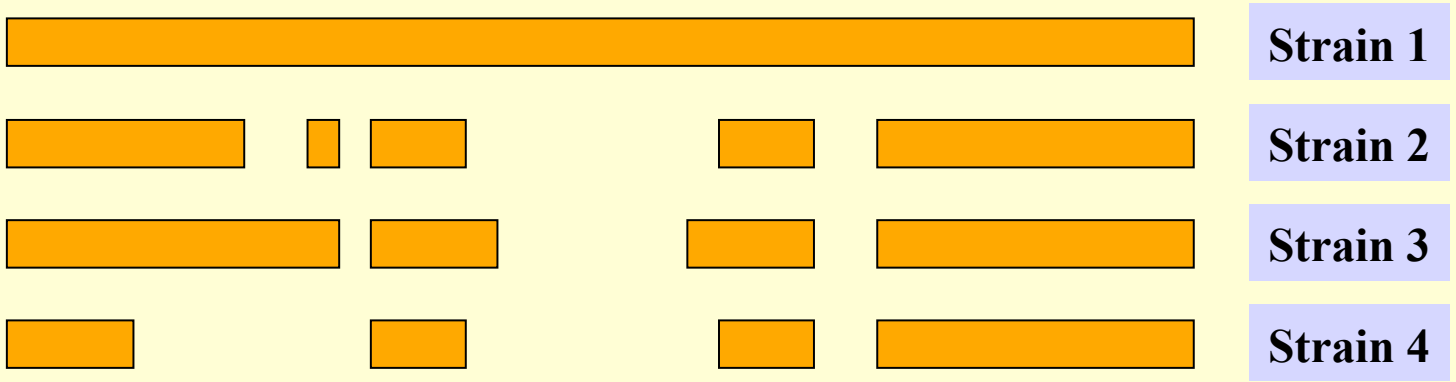
- Program(s) to search all sequence databases
- Tremendous Speed/Less Sensitive
- Statistical Significance reported
- WWWBLAST, QBLAST (send now, retrieve results later), Standalone BLAST, BLASTcl3 (Client version, TCP/IP connection to NCBI server), BLAST URLAPI (to access QBLAST, no local client)

# BLAST Strategy & Improvements

- Lipman et al.: speeded up finding "runs" of "hot spots".
- Eugene Myers '94: "Sublinear algorithm for approximate keyword matching".
- Karlin, Altschul, Dembo '90, '91: "Statistical Significance of Matches"

# Why Gaps?

- Example: Aligning HIV sequences.



# BLAST Variants

- **Nucleotide BLAST**
  - **Standard blastn**
  - **MEGABLAST** (Compare large sets, Near-exact searches)
  - **Short Sequences** (higher E-value threshold, smaller word size, no low-complexity filtering)
- **Protein BLAST**
  - **Standard blastp**
  - **PSI-BLAST** (Position Specific Iterated BLAST)
  - **PHI-BLAST** (Pattern Hit Initiated BLAST; reg expr. Or Motif search)
  - **Short Sequences** (higher E-value threshold, smaller word size, no low-complexity filtering, PAM-30)
- **Translating BLAST**
  - **Blastx**: Search nucleotide sequence in protein database (6 reading frames)
  - **Tblastn**: Search protein sequence in nucleotide dB
  - **Tblastx**: Search nucleotide seq (6 frames) in nucleotide DB (6 frames)

# BLAST Cont'd

- **RPS BLAST**
  - Compare protein sequence against Conserved Domain DB; Helps in predicting rough structure and function
- **Pairwise BLAST**
  - blastp (2 Proteins), blastn (2 nucleotides), tblastn (protein-nucleotide w/ 6 frames), blastx (nucleotide-protein), tblastx (nucleotide w/6 frames-nucleotide w/ 6 frames)
- **Specialized BLAST**
  - Human & Other finished/unfinished genomes
  - *P. falciparum*: Search ESTs, STSs, GSSs, HTGs
  - VecScreen: screen for contamination while sequencing
  - IgBLAST: Immunoglobin sequence database

# BLAST Credits

- Stephen Altschul
- Jonathan Epstein
- David Lipman
- Tom Madden
- Scott McGinnis
- Jim Ostell
- Alex Schaffer
- Sergei Shavirin
- Heidi Sofia
- Jinghui Zhang

# Databases used by BLAST

- **Protein**
  - nr (everything), swissprot, pdb, alu, individual genomes
- **Nucleotide**
  - nr, dbest, dbsts, htgs (unfinished genomic sequences), gss, pdb, vector, mito, alu, epd
- **Misc**

# Rules of Thumb

- Most sequences with significant similarity over their entire lengths are homologous.
- Matches that are > 50% identical in a 20-40 aa region occur frequently by chance.
- Distantly related homologs may lack significant similarity. Homologous sequences may have few absolutely conserved residues.
- A homologous to B & B to C $\Rightarrow$ A homologous to C.
- Low complexity regions, transmembrane regions and coiled-coil regions frequently display significant similarity without homology.
- Greater evolutionary distance implies that length of a local alignment required to achieve a statistically significant score also increases.

# Rules of Thumb

- Results of searches using different scoring systems may be compared directly using normalized scores.
- If S is the (raw) score for a local alignment, the **normalized** score S' (in bits) is given by

$$S' = \frac{\lambda - \ln(\mathrm{K})}{\ln(2)}$$

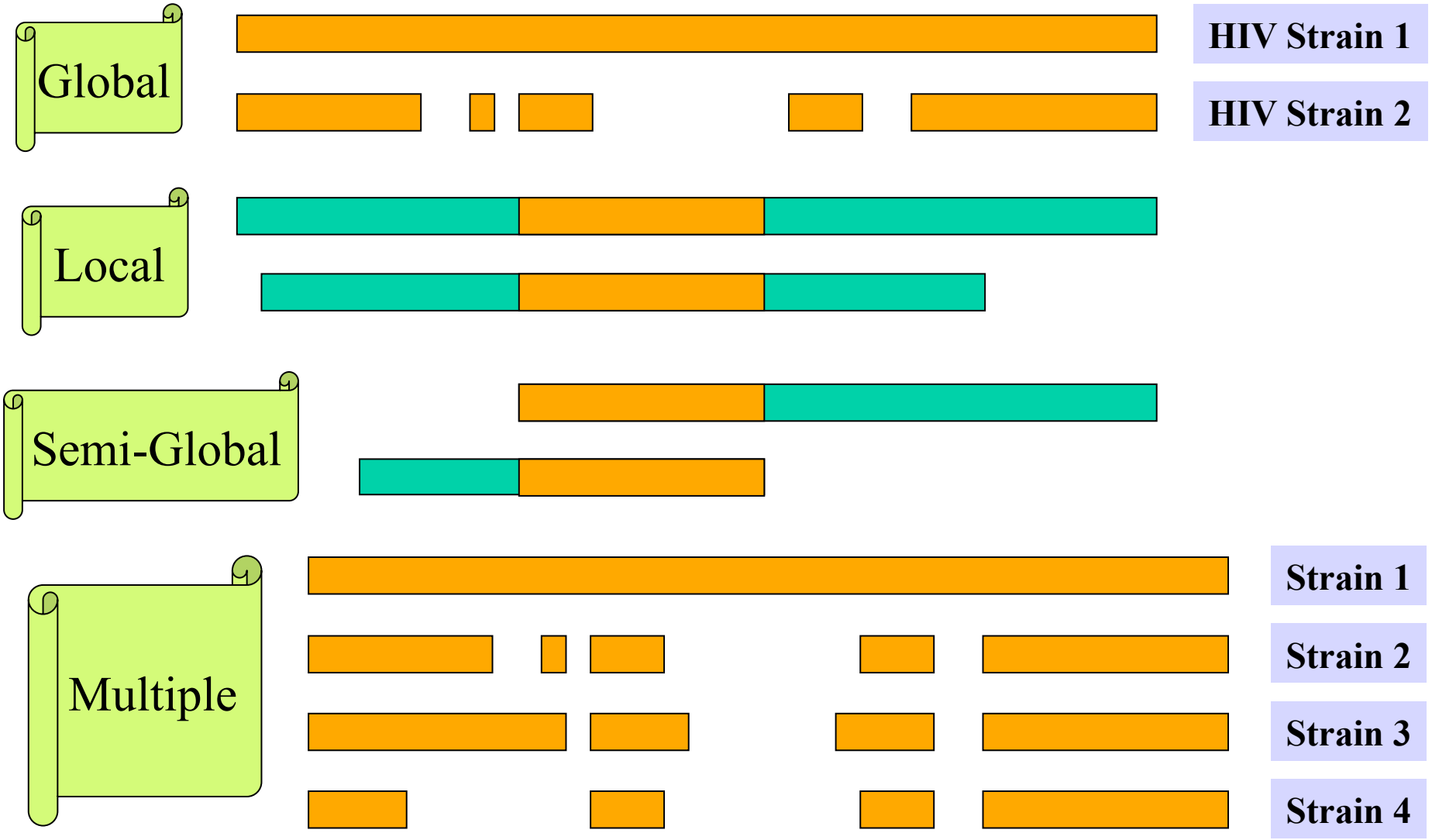
The parameters depend on the scoring system.

- **Statistically significant normalized score**,

$$S' > \log\left(\frac{N}{E}\right)$$

where E-value = E, and N = size of search space.

# Types of Sequence Alignments



# Global Alignment: An example

```
V: G A A T T C A G T T A
W: G G A T C G A
```

| | | G | A | A | T | T | C | A | G | T | T | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 0 | | | | | | | | | | | |
| G | 0 | | | | | | | | | | | |
| A | 0 | | | | | | | | | | | |
| T | 0 | | | | | | | | | | | |
| C | 0 | | | | | | | | | | | |
| G | 0 | | | | | | | | | | | |
| A | 0 | | | | | | | | | | | |

**Given**

$\delta[I, J]$ = Score of Matching
the $I^{th}$ character of sequence V &
the $J^{th}$ character of sequence W

**Compute**

$S[I, J]$ = Score of Matching
First $I$ characters of sequence V &
First $J$ characters of sequence W

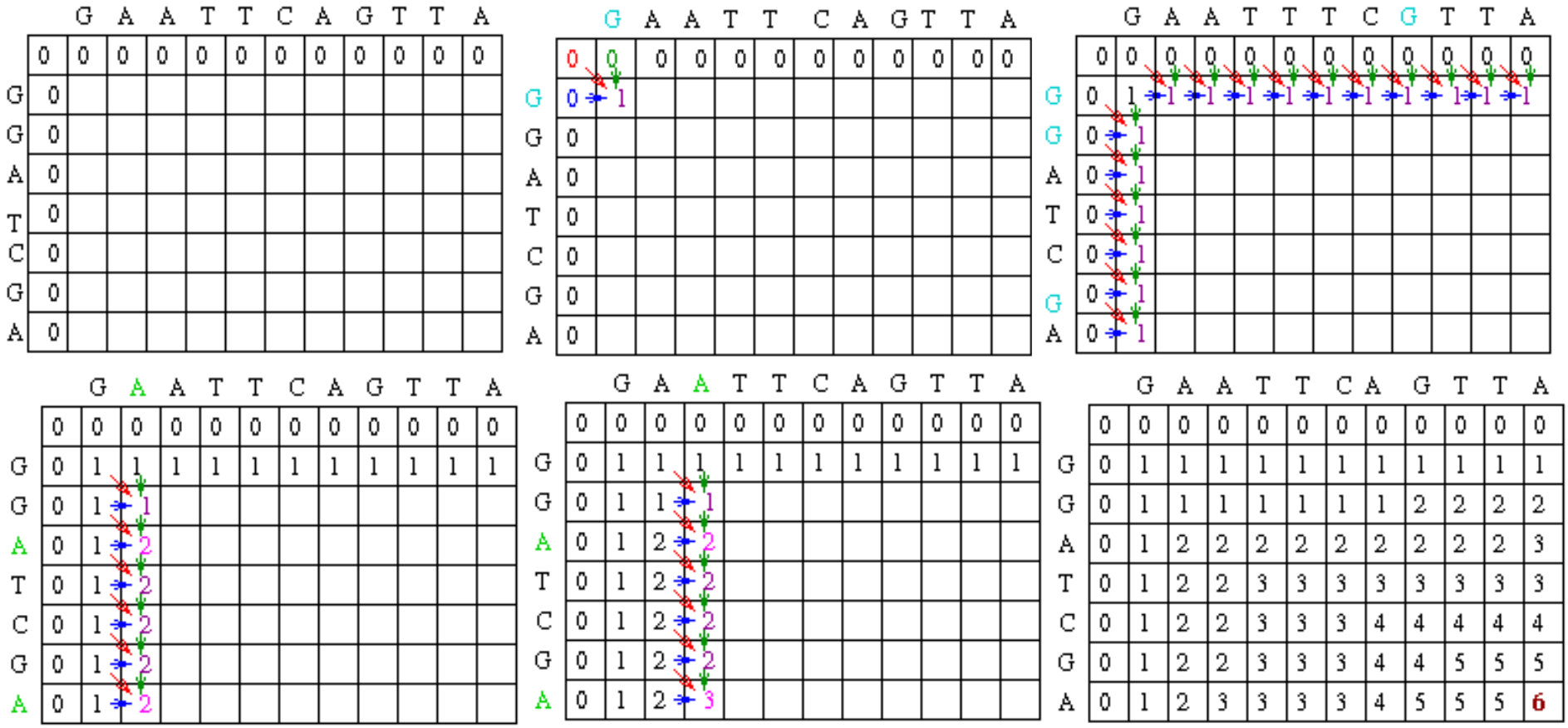
**Recurrence Relation**

$S[I, J] = MAXIMUM \{$
$S[I-1, J-1] + \delta(V[I], W[J])$,
$S[I-1, J] + \delta(V[I], —)$,
$S[I, J-1] + \delta(— , W[J]) \}$

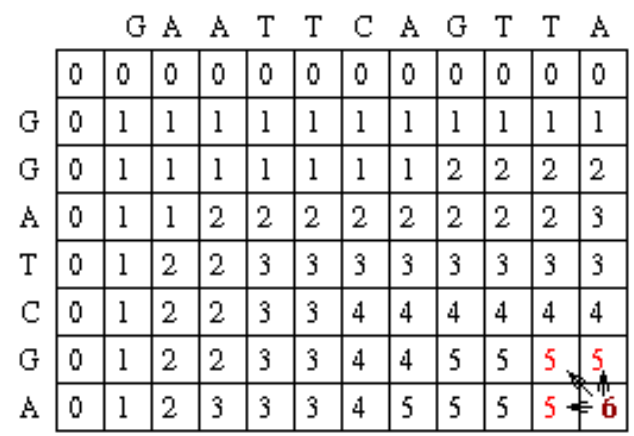
# Global Alignment: An example

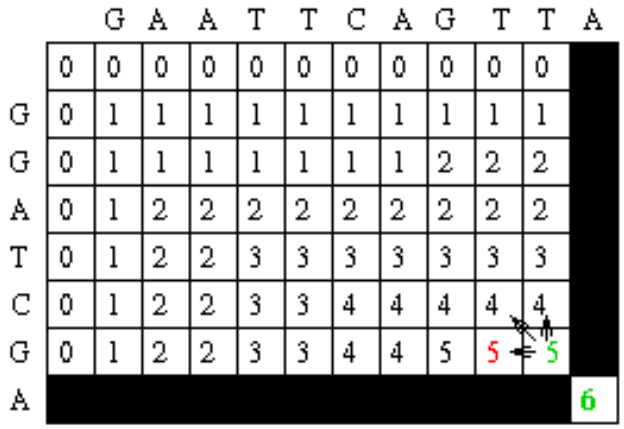
$S[I, J] = MAXIMUM \{$
$S[I-1, J-1] + \delta(V[I], W[J]),$
$S[I-1, J] + \delta(V[I], —),$
$S[I, J-1] + \delta(— , W[J]) \}$

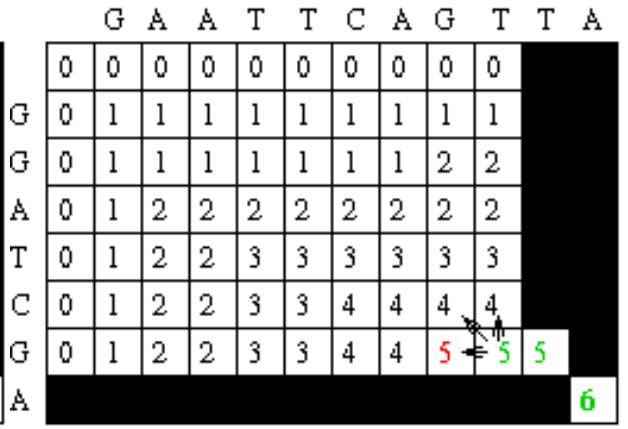
```
V: G A A T T C A G T T A
W: G G A T C G A
```

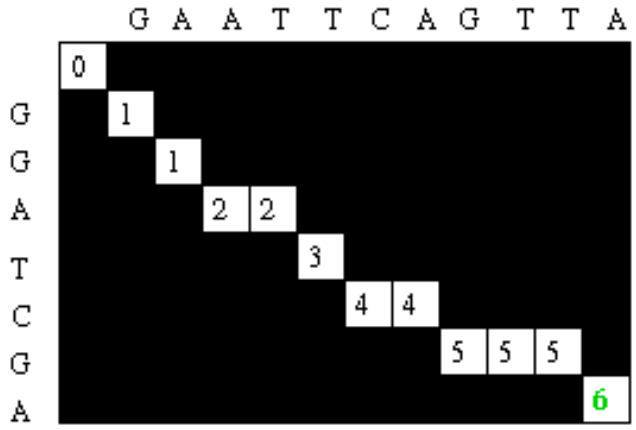


# Traceback



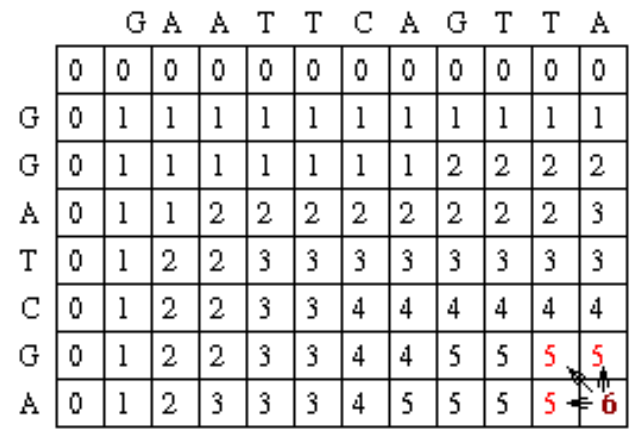



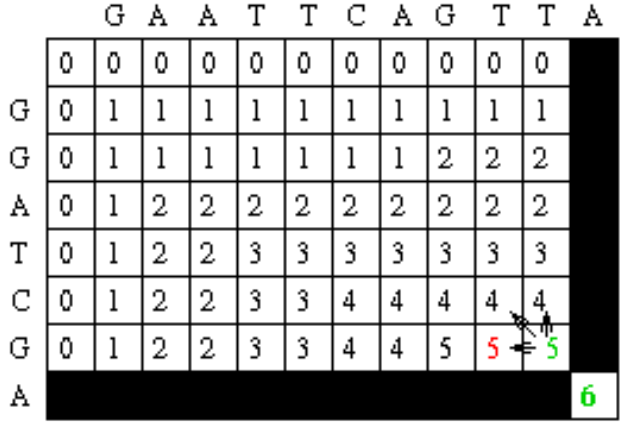



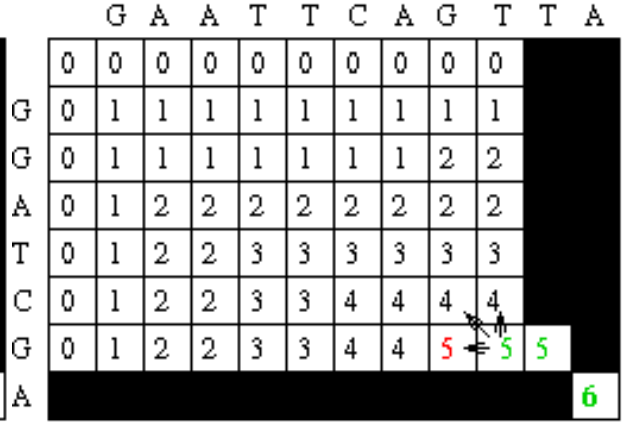



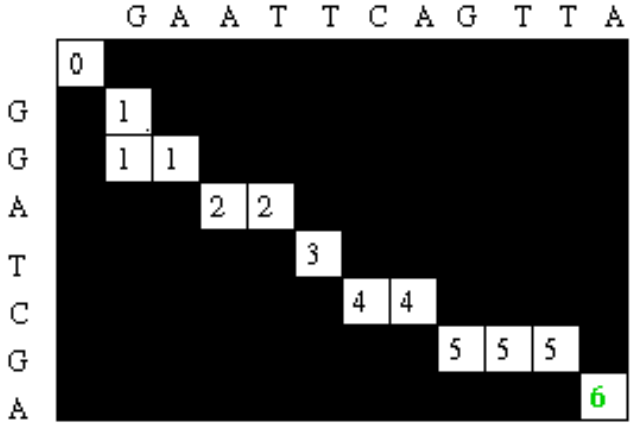

```
V: G A A T T C A G T T A
   |   |   |  |   |     |
W: G G A - T C - G - - A
```

# Alternative Traceback




```
V: G - A A T T C A G T T A
   |   |   | |   |     |
W: G G - A - T C - G - - A
```

```
V: G A A T T C A G T T A
   |   |   | |   |     |
W: G G A - T C - G - - A
```

Previous

# Improved Traceback

| | | G | A | A | T | T | C | A | G | T | T | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 0 | ×1 | ←1 | ← 1 | ← 1 | ← 1 | ← 1 | ← 1 | ×1 | ← 1 | ← 1 | ← 1 |
| G | 0 | ×1 | ↑1 | ↑1 | ↑1 | ↑1 | ↑1 | ↑1 | ×2 | ← 2 | ← 2 | ← 2 |
| A | 0 | ↑1 | ↑1 | ×2 | ← 2 | ← 2 | ← 2 | ×2 | ↑2 | ↑2 | ↑2 | ×3 |
| T | 0 | ↑1 | ← 2 | ↑2 | ×3 | ×3 | ← 3 | ← 3 | ← 3 | ×3 | ×3 | ↑3 |
| C | 0 | ↑1 | ↑2 | ↑2 | ↑3 | ↑3 | ×4 | ← 4 | ← 4 | ← 4 | ← 4 | ← 4 |
| G | 0 | ↑1 | ↑2 | ↑2 | ↑3 | ↑3 | ↑4 | ↑4 | ×5 | ← 5 | ← 5 | ← 5 |
| A | 0 | ↑1 | ↑2 | ×3 | ↑3 | ↑3 | ↑4 | ×5 | ↑5 | ↑5 | ↑5 | ×6 |

# Improved Traceback

| | | G | A | A | T | T | C | A | G | T | T | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 0 | ×1 | ←1 | ← 1 | ← 1 | ← 1 | ← 1 | ← 1 | ×1 | ← 1 | ← 1 | ← 1 |
| G | 0 | ×1 | ↑1 | ↑1 | ↑1 | ↑1 | ↑1 | ↑1 | ×2 | ← 2 | ← 2 | ← 2 |
| A | 0 | ↑1 | ↑1 | ×2 | ← 2 | ← 2 | ← 2 | ×2 | ↑2 | ↑2 | ↑2 | ×3 |
| T | 0 | ↑1 | ← 2 | ↑2 | ×3 | ×3 | ← 3 | ← 3 | ← 3 | ×3 | ×3 | ↑3 |
| C | 0 | ↑1 | ↑2 | ↑2 | ↑3 | ↑3 | ×4 | ← 4 | ← 4 | ← 4 | ← 4 | ← 4 |
| G | 0 | ↑1 | ↑2 | ↑2 | ↑3 | ↑3 | ↑4 | ↑4 | ×5 | ← 5 | ← 5 | ← 5 |
| A | 0 | ↑1 | ↑2 | ×3 | ↑3 | ↑3 | ↑4 | ×5 | ↑5 | ↑5 | ↑5 | ×6 |

# Improved Traceback

| | | G | A | A | T | T | C | A | G | T | T | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 0 | ×1 | ←1 | ← 1 | ← 1 | ← 1 | ← 1 | ← 1 | ×1 | ← 1 | ← 1 | ← 1 |
| G | 0 | ×1 | ↑1 | ↑1 | ↑1 | ↑1 | ↑1 | ↑1 | ×2 | ← 2 | ← 2 | ← 2 |
| A | 0 | ↑1 | ↑1 | ×2 | ← 2 | ← 2 | ← 2 | ×2 | ↑2 | ↑2 | ↑2 | ×3 |
| T | 0 | ↑1 | ← 2 | ↑2 | ×3 | ×3 | ← 3 | ← 3 | ← 3 | ×3 | ×3 | ↑3 |
| C | 0 | ↑1 | ↑2 | ↑2 | ↑3 | ↑3 | ×4 | ← 4 | ← 4 | ← 4 | ← 4 | ← 4 |
| G | 0 | ↑1 | ↑2 | ↑2 | ↑3 | ↑3 | ↑4 | ↑4 | ×5 | ← 5 | ← 5 | ← 5 |
| A | 0 | ↑1 | ↑2 | ×3 | ↑3 | ↑3 | ↑4 | ×5 | ↑5 | ↑5 | ↑5 | ×6 |

```
V: G A - A T T C A G T T A
   |   |   |   |   |     |
W: G - G A - T C - G - - A
```